Case report

mTORC Pathway Activation and Effect of Sirolimus on Native Kidney Antiphospholipid Syndrome Nephropathy

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ABSTRACT

Despite optimal anticoagulation and blood pressure control, patients with antiphospholipid syndrome (APS) nephropathy frequently progress to end-stage kidney disease, and recurrence after transplantation is common. The mechanistic target of rapamycin complex (mTORC) pathway was recently identified as a potential intermediate and a therapeutic target in vascular lesions associated with APS nephropathy. However, these results were derived from the retrospective analysis of a small cohort of patients receiving sirolimus after kidney transplantation. Therefore, they warranted external validation and the demonstration of the potential benefit of sirolimus in native kidneys APS nephropathy.

Here we report on the case of a patient with active lesions of APS nephropathy occurring on native kidneys, in which endothelial mTORC activation was substantiated at the molecular level. Treatment with sirolimus successfully inhibited AKT/mTORC pathway on a repeat kidney biopsy, and was associated with significant improvement of kidney function and lesions of vasculopathy. Drug tolerance was excellent during the whole follow-up.

This case validates and extends previous observations in kidney transplant recipients, and demonstrates that endothelial activation of the AKT/mTORC pathway occurs in the damaged renal vasculature of native kidneys in APS nephropathy. These findings further support the potential of a precision medicine, and the use of mTORC activation as a biomarker of disease activity and as therapeutic target in patients with APS nephropathy.

INTRODUCTION

The antiphospholipid syndrome (APS) is an autoimmune disease defined by the presence of circulating antiphospholipid antibodies, and vascular thrombosis or obstetrical complications [1]. Additional, non-criteria features of APS include thrombocytopenia, heart valve disease, and cutaneous, neurologic, and renal manifestations.

Kidney involvement in APS is associated with a large spectrum of clinicopathological expressions, ranging from large vessel thrombosis to lesions affecting the renal microarchitecture, a condition known as APS nephropathy [2, 3]. The prevalence of APS nephropathy is estimated at 3-9% of primary and 9-44% of secondary APS [2]. However, it is probably largely underestimated as it may occur in patients with antiphospholipid antibodies who lack the criteria for definite APS [2]. Typically, APS nephropathy manifests as a slowly progressive vascular nephropathy, with hypertension, reduced glomerular filtration rate, and mild to moderate proteinuria [4].

Recently, the mechanistic target of rapamycin complex (mTORC) pathway was identified as a potential intermediate and a therapeutic target in chronic vascular lesions associated with APS nephropathy [5]. Mechanistically, the authors demonstrated endothelial activation of the mTORC pathway in the renal microvasculature of patients with APS nephropathy, and its association with the proliferation of both endothelial and vascular smooth muscle cells. They also suggested mTOR inhibitor sirolimus reduces the risk of recurrence and improves allograft survival in patients with APS nephropathy who required kidney transplantation [5].

Although they provided novel insights into the pathophysiology of the disease, these results were derived from the retrospective analysis of a small cohort of patients receiving sirolimus after kidney transplantation. As a result, they warranted external validation and prospective studies to definitely establish the potential of mTORC activation as a biomarker

and a therapeutic target in APS nephropathy. Furthermore, the potential clinical benefit of sirolimus for patients with APS nephropathy on native kidneys still remained unknown.

Here we report on the case of a patient with chronic active lesions of APS nephropathy occurring on native kidneys, in which endothelial mTORC activation was substantiated at the molecular level. Treatment with sirolimus successfully inhibited AKT/mTORC pathway on a repeat kidney biopsy, and was associated with improvement of vasculopathy and kidney function.

CASE REPORT

A 36-year-old Caucasian woman was referred for the evaluation of recent decline in kidney function. Previous medical history was remarkable because of acute bowel ischemia secondary to mesenteric vein thrombosis at the age of 22. Screening for antiphospholipid antibodies showed the presence of significant levels of anti-β2-glycoprotein I antibodies and a lupus anticoagulant, confirmed on a second sample. The search for anti-nuclear and anti-DNA antibodies and for human immunodeficiency virus infection returned negative, and she was diagnosed with primary APS. Mild hypertension was also observed at that time; kidney function was normal (serum creatinine level 0.96 mg/dl, CKD-EPI eGFR 84 ml/min/1.73 m^2). Oral anticoagulation with vitamin K antagonist was started, and later replaced by rivaroxaban (at the age of 34) because of decreased risk of bleeding and increased convenience with the latter. Blood pressure control was achieved with angiotensin converting enzyme inhibitor ramipril. At the age of 35, she developed chest pain associated with elevation of troponin (troponin T, up to 89 pg/ml; normal range, <15), normal cardiac ultrasound and coronary angiography, suggesting microvascular heart disease. Concomitantly, serum creatinine raised from 1.27 mg/dl (eGFR 48 ml/min/1.73 m^2) to 1.84

mg/dl (eGFR 35 ml/min/1.73 m²) over a few months (Table 1), and she was referred for additional evaluation.

At the outpatient clinic, blood pressure was 154/108 mmHg; the rest of physical examination was unremarkable. Urinary sediment was bland and proteinuria <0.3 g/day. Ultrasound showed normal-sized kidneys, without renal artery thrombosis. The presence of significant titers of circulating anti-β2-glycoprotein I antibodies (IgG, 17.0 U/mL, and IgM, 13.3 U/mL; normal range <7) and a lupus anticoagulant were confirmed; the search for anti-cardiolipin antibodies returned negative. There was no biological evidence of thrombotic microangiopathy (Table 1).

A first kidney biopsy was performed. Small and medium-sized interlobular arteries exhibited fibrous intimal hyperplasia, onion-skin arrangement, vascular lumen narrowing and intravascular thrombosis (Fig 1). Glomerular capillaries showed endothelial cell swelling and focal lesions of thrombotic microangiopathy (Fig S1). Interstitial fibrosis/tubular atrophy scoring was 1, on a scale from 0 to 3 [6] (Fig S2). There was no evidence of immunoglobulin or complement deposition. We used confocal microscopy to investigate potential mTORC pathway activation, and evaluated the phosphorylation of two critical downstream targets, S6 ribosomal protein (S6RP) and AKT on Ser^{473}, in the renal vascular endothelium. Immunofluorescence studies revealed multiple sections in arterioles positive for P-S6RP and P-AKT (Ser^{473}) (Fig 1, Fig S3). Altogether, these findings were consistent with the diagnosis of APS nephropathy and substantiated AKT/mTORC pathway activation in endothelial cells lining damaged renal vessels.

Based on these results, and because of the progressive decline in kidney function despite anticoagulation and treatment with ramipril, mTOR inhibitor sirolimus was introduced at the dose of 2 mg o.d., and further adapted to achieve through levels of 4-5 ng/ml (Table 1). Treatment with sirolimus was associated with a significant improvement in

kidney function, and serum creatinine level dropped from 1.84 mg/dl (eGFR 35 ml/min/1.73 m²) to 1.30 mg/dl (eGFR 53 ml/min/1.73 m²) after 3 months. At month 6, the beneficial effect of sirolimus was maintained, with a creatinine level of 1.36 mg/dl (eGFR 50 ml/min/1.73 m²), and a repeat kidney biopsy was performed. The second kidney biopsy showed resolution of acute microangiopathy, with residual intimal fibrosis in an arteriole (Fig 1). Interstitial fibrosis/tubular atrophy scoring remained stable as compared with the first kidney biopsy (Fig S2). Importantly, immunofluorescence studies showed the inactivation of the AKT/mTORC pathway in endothelial cells under treatment with sirolimus, with absence of P-S6RP and P-AKT (Ser^{473}) expression in vascular sections (Fig 1). Drug tolerance was excellent during the whole follow-up.

DISCUSSION

In the present case, we first demonstrated endothelial mTORC activation in the native kidneys of a patient with APS nephropathy, extending previous observations from allograft recipients. Next, we showed that treatment with sirolimus successfully inhibited the AKT/mTORC pathway in the renal vasculature, and led to significant improvement of the vasculopathy and kidney function. To the best of our knowledge, this is the first time sirolimus is used to treat APS nephropathy in a non-transplant recipient, and that longitudinal follow-up, including repeat kidney biopsy, validates the critical role of AKT/mTORC activation in APS nephropathy.

mTOR is a kinase that integrates a variety of signaling pathways to regulate cell growth, proliferation, and survival [7, 8]. mTOR forms two functionally distinct complexes, mTORC1 and mTORC2, which act *via* phosphorylation of S6 ribosomal protein (S6RP) and phosphorylation of AKT on Ser^{473}, respectively. Data from translational studies demonstrated

that the mTORC pathway is activated in the vascular endothelium of proliferating intrarenal vessels from patients with APS nephropathy [5]. *In vitro*, immunoglobulin G antibodies from patients with APS reproduced such activation, which was mediated by the phosphatidyl-inositol 3-kinase-AKT pathway. In the subset of patients with APS nephropathy who required transplantation, the use of sirolimus as part of the immunosuppressive regimen was associated with lower rate of vascular lesions and better renal outcome as compared with patients not receiving the mTOR inhibitor. In our patient, the native kidney biopsy performed at the time of progressive kidney dysfunction showed typical vascular lesions of APS nephropathy, and provided evidence for endothelial mTORC activation in the renal vasculature. Treatment with sirolimus inhibited mTORC activation, dramatically reduced signs of vasculopathy, and significantly improved kidney function.

The beneficial effects of sirolimus were observed on top of systemic anticoagulation and inhibition of the renin angiotensin system. Efficient oral anticoagulation remains the cornerstone treatment for patients with APS and venous thromboembolism, although there is no evidence of efficacy in APS nephropathy. In a randomized open label trial comparing rivaroxaban with warfarin in APS with previous venous thromboembolism, no patient in either group had bleeding or thrombosis during the 6-month follow-up period, suggesting rivaroxaban could be an effective and safe alternative in APS [9]. In those patients, rivaroxaban also reduced complement activation as compared to warfarin, potentially providing an additional benefit to its anticoagulant effect [10]. In addition to systemic anticoagulation, pharmacological blockade of the renin angiotensin system is useful to achieve blood pressure control and was suggested to confer additional reno-protection, beyond antihypertensive and antiproteinuric effects, in patients with APS nephropathy [11, 12]. Despite the use of anticoagulation and renin angiotensin system blockade, patients with APS nephropathy frequently progress to end-stage kidney disease, and the disease may recur

after transplantation, leading to loss of the allograft [2, 11, 13]. In this context, further evidence supporting the beneficial effects of mTOR inhibition in APS nephropathy is of course welcome.

Although sirolimus appears as a promising, effective and safe targeted therapy for patients with APS nephropathy, it is important to remind the potential risk of drug-drug interactions and side effects of mTORC inhibition, especially in patients with severe chronic kidney disease. Potential side effects of sirolimus include pneumonitis, impaired wound healing, apthous ulcers, glucose intolerance, dyslipidemia and proteinuria. The AKT/mTORC pathway constitutes a critical stress-response component in podocytes and prevents cytoskeleton alterations and apoptosis after nephron reduction [14]. In the setting of severely impaired kidney function, the use of sirolimus is associated with an increased risk of glomerular lesions, albuminuria, and progression of kidney disease. Currently, it seems reasonable to consider the use or to continue treatment with sirolimus only in patients with non-severe CKD (eGFR >30 ml/min/1.73 m^2). There is no 'optimal dose' of sirolimus for the treatment of APS nephropathy, and future studies will need address this issue – to ensure therapeutic efficiency while avoiding side effects. In our patient, regular therapeutic drug monitoring was applied to achieve trough levels close to those used in kidney transplant recipients [15].

In conclusion, we validated and extended previous observations in kidney transplant recipients, and demonstrated that endothelial activation of the AKT/mTORC pathway occurs in the damaged renal vasculature of native kidneys in APS nephropathy. Treatment with sirolimus successfully inhibited mTORC activation, dramatically reduced renal vasculopathy, and improved kidney function. These findings further support the potential of a precision medicine for patients with APS nephropathy, and the use of mTORC activation as a biomarker of disease activity and as therapeutic target.

COMPETING INTERESTS

The authors declare no competing interests.

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SUPPLEMENTARY MATERIAL

Item S1: Materials and methods.

Fig S1: Focal lesions of thrombotic microangiopathy on the first kidney biopsy.

Fig S2: Low magnification images of kidney biopsies.

Fig S3: Negative controls for immunofluorescence assays.

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FIGURE LEGEND

Figure 1. mTORC pathway activation and effect of sirolimus on native kidney APS nephropathy.

(A-B) Trichrome staining showed alterations of the renal microvasculature at presentation, before sirolimus initiation, including thickening of glomerular capillary walls (A), and severe fibrous intimal hyperplasia with a chronic, organized thrombus in a renal arteriole (B).

(C-D) Confocal microscopy on the same kidney biopsy showed positive staining for P-S6 ribosomal protein (S6RP, red, C) and P-Akt (Ser^{473}, red, D) in vascular sections positive for α-smooth muscle actin (α-SMA, green, C) or von Willebrand factor (vWF, green, D), demonstrating the recruitment of mTORC1 and mTORC2, respectively, in the damaged renal vasculature.

(E-H) After 6 months of sirolimus, a repeat kidney biopsy showed the resolution of vascular damage both in glomerular capillaries (E) and renal arterioles (F), and absence of phosphorylated S6RP (G) or Akt (Ser^{473}) in vascular sections positive for α-smooth muscle actin (α-SMA, green, G) or von Willebrand factor (vWF, green, H).

TABLE

Table 1. Laboratory tests.

Lab test	Units	Normal range	6 mo. before	3 mo. before	Sirolimus initiation	3 mo. after	6 mo. after
Serum creatinine	mg/dL	0.60-1.30	1.27	1.65	1.84	1.30	1.36
eGFR	mL/min/1.73 m²	>60	48	40	35	53	50
Hemoglobin	g/dL	12.2-15.0	10.5	11.6	12.3	11.2	13.6
Platelet count	$10^3/\mu L$	150-450	199	179	206	264	221
Haptoglobin	g/L	0.3-2	0.63	0.91	0.76	0.96	0.86
Lactate dehydrogenase	IU/L	<250	258	240	262	291	240
Schistocystes	%	<1	<1	<1	<1	<1	<1
C3	g/L	0.9-1.8	1.4	0.8	0.8	0.8	1.0
Total bilirubin	mg/dL	<1.2	0.4	0.5	0.5	0.4	0.5
Sirolimus trough level	ng/mL	4.0-15.0	-	-	-	4.6	4.4
Total cholesterol	mg/dL	<190	155	227	251	240	243
LDL cholesterol	mg/dL	<115	80	93	115	105	109
Triglycerides	mg/dL	<150	136	75	87	78	90
Fasting blood glucose	mg/dL	<100	-	-	83	81	92
UPCR	g/g	<0.15	0.16	0.18	0.25	0.31	0.49

Abbreviations: eGFR, estimated glomerular filtration rate calculated using the CKD-EPI equation; UPCR, urinary protein to creatinine ratio; mo., months.